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TECHNICAL MANUSCRIPT 534

HYDROLYTIC ENZYMES IN THE NEONATAL LUNG

John R. Esterly
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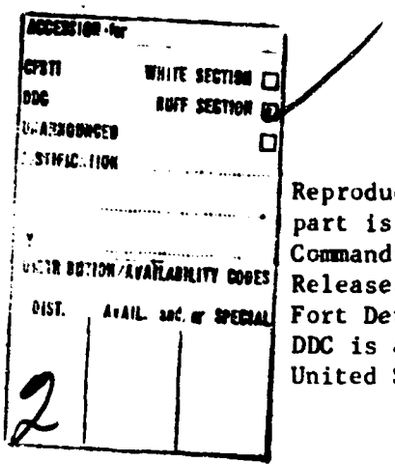
JULY 1969

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland



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HYDROLYTIC ENZYMES IN THE NEONATAL LUNG

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Pathology Division
MEDICAL SCIENCES LABORATORIES

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July 1969

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ABSTRACT

Histochemical methods were used to study the development of representative enzymes associated with lysosomal activity in sections of the lung from late fetal, neonatal, and adult rats. Staining for β-glucuronidase and acid phosphatase was present in the fetus and following birth, and the number and intensity of reactive cells increased to adult levels by 7 to 10 days of life. Reactions for β-galactosidase were not found in newborn animals, but moderate staining was seen by the 14th day. In contrast to these lysosomal enzymes, high levels of cytochrome oxidase staining were found in all specimens and no age-dependent changes were seen. Acid phosphatase activity was present in nearly all cells in the adult lung, but the indolyl reactions for the glycosidases were localized to single cells. In spite of this resolution, it was not possible to be certain of the identity of many of the reactive cells. The majority appeared to be type II epithelial cells. Alveolar macrophages were usually reactive but relatively few such cells were found, especially in the neonatal specimens. The results of this and other studies indicate that these cells have many common metabolic pathways. Other data, however, suggest a dissimilar origin and function of type II cells and macrophages.

I. INTRODUCTION*

The metabolism of alveolar macrophages has been studied in detail during the past decade,[1,2] and these data have been important in our understanding of factors in host resistance to tuberculosis and other airborne infections.[3] This is especially true of the hydrolases that participate in the digestion of phagocytosed material.[1,4,5] Macrophages from lung washings have been used in these studies and considerably less is known about the distribution of activity for these enzymes in other pulmonary cells. In spite of the technical difficulties of working with lung sections and the semi-quantitative limitations, enzyme histochemistry provides the advantage of localizing and comparing reactivity among tissue components. The objective of the present study was to trace the development of several hydrolytic enzymes and to detect potential changes in the localization of their activity during the neonatal period.

II. MATERIALS AND METHODS

The specimens were obtained from groups of four to six inbred Fischer 344 rats at each of the following ages: 19 and 21 days gestation, newborn, 4, 7, 10, 14, and 28 days, and 6 months. Half-centimeter slices of each lung were obtained from sacrificed animals and quickly frozen in a dry ice - acetone bath and stored at -70 C. Except for the fetal and newborn animals, several specimens from each group were inflated with gelatin prior to freezing.[6] Frozen sections were subsequently cut and incubated. Halogen-substituted indolyl glycosides were used for β-D-glucuronidase;[7,8] the AS-BI method was used for acid phosphatase at pH 5.2.[9] Cytochrome oxidase was also demonstrated as a control of enzyme preservation,[10] and strong reactions were found in all specimens.

Sections from each group were lightly counterstained with hematoxylin or nuclear Fast Red. Because the reaction product formed by the indolyl substrates is stable and insoluble, it was possible to counterstain these sections with the periodic acid - Schiff sequence. Portions of lung from each age group were also processed routinely and paraffin sections were stained with hematoxylin and eosin and the periodic acid - Schiff method. All specimens were screened to exclude the presence of inflammatory infiltrates.

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The sections were arbitrarily graded (0 to ++++) by comparison with the specimen with the strongest reaction for each technique. Only small variations were seen among the specimens within age groups and, for each substrate, there were usually parallel changes in the intensity of staining and in the number of reactive cells.

III. OBSERVATIONS

The histologic pattern of development in the specimens was similar to that found in man and other mammals. By late gestation, cellular morphology and organization of the airways was relatively mature. The lobular patterns in the fetal and neonatal animals resembled those in the adult rat lung. The alveolar epithelium was attenuated and there was abundant vascularization of alveolar septa. The most striking change in the older animals was the presence of diffuse, peribronchial lymphoid aggregates, which in the adult had the more differentiated pattern of lymph nodes. Presumptive type II cells were recognized by their morphology and location, but in any given instance, identification could not be made with certainty. In neither the routine nor the frozen sections were granules definitely reactive to periodic acid - Schiff visualized in type II cells.

Galactosidase reactions were absent in the fetal and newborn specimens. There were weak reactions in rare cells at 7 and 10 days of age; thereafter, the number of reactive cells and their intensity of staining increased progressively. The strongest reactions were found in the adult animals (Table 1).

The reaction product appeared as blue-green rods and granules localized in the cytoplasm of single epithelial cells and occasional macrophages within alveoli. In addition, foci of diffuse staining were sometimes seen over alveolar septa. (It is unlikely that such areas represent enzyme diffusion, because they were not usually adjacent to strongly reactive cells, and they were also found in specimens prefixed in 10% formol calcium for 30 minutes at 4 C.) The location and shape of the majority of reactive cells were compatible with type II cells (Fig. 1). Endothelial cells were unreactive, but staining was seen in occasional bronchial epithelial cells and in reticular cells in the peribronchial lymphoid aggregates and lymph nodes.

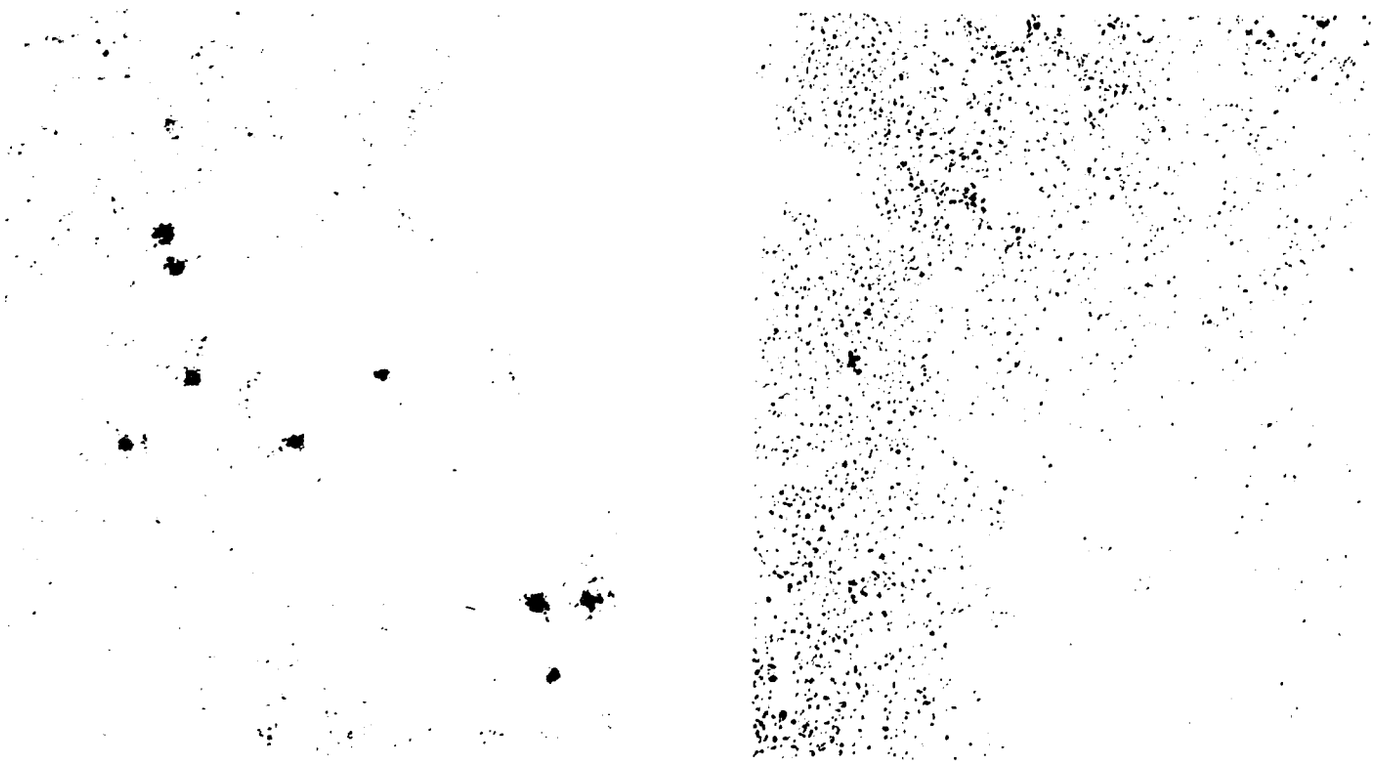
Reactions indicative of glucuronidase activity were found in all specimens. In the fetal lung, stained cells were inconspicuous, but the frequency and intensity of staining increased rapidly in the age samples examined (Fig. 2). In general, the reactions were stronger than those for galactosidase, but the quality and localization of the reaction product were otherwise indistinguishable.

TABLE 1. REACTIVITY OF HYDROLYTIC ENZYMES IN THE LUNG OF THE DEVELOPING RAT[a]

Age	β-Galactosidase	β-Glucuronidase	Acid Phosphatase
19-day fetus	0	0 to +	+ to ++
21-day fetus	0	+	+ to ++
Newborn	0	++	++
4 days	0	+ to ++	++
7 days	0 to +	++	+++
10 days	0 to +	+++	+++
14 days	++	+++ to ++++	++++
28 days	+++	++++	++++
6 months	++++	++++	++++

a. The relative activity of the specimens was graded by comparison with the most reactive sections for each technique. The estimate of staining at each age includes changes both in the number and in the intensity of reactive cells.

FIGURE 1. Beta-Galactosidase Activity Localized to the Cytoplasm of Single Alveolar Lining Cells and Occasional Macrophages Within Alveoli. The morphology and position of the reactive epithelial cells are compatible with those of type II cells. Indolyl galactoside with hematoxylin counterstain. A, 14-day; B and C, adult rat. (160X)



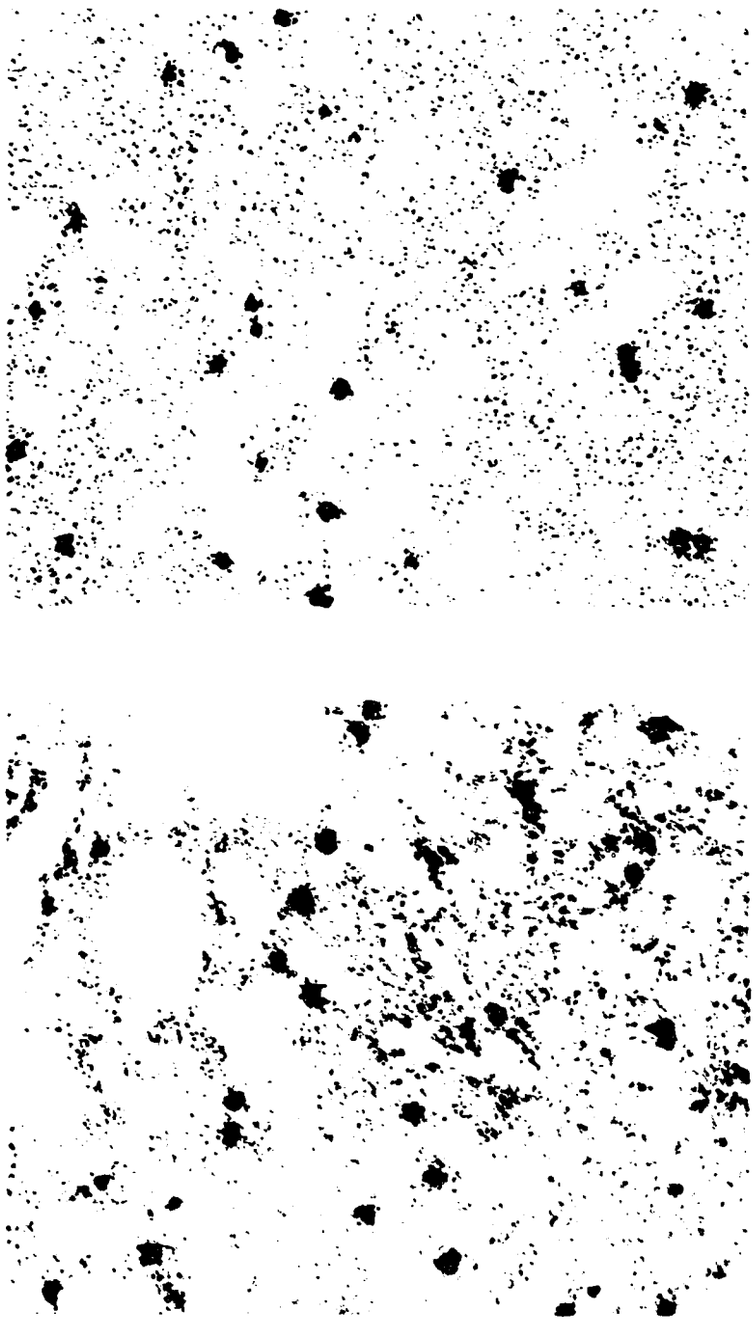


FIGURE 2. Beta-Glucuronidase Staining Present in Fetal and Newborn Specimens and Rapidly Increased Reactivity to Adult Levels by the Second Week of Life. Indolyl glucuronide with hematoxylin counterstain. A, 19-day fetus; B, newborn; C, 14-day; D, adult rat. (Uninflated, 100X)

The diazonium salt in the acid phosphatase reaction appeared as fairly uniform red granules. In the 28-day-old and 6-month-old animals, diffuse pink cytoplasmic staining was also present in some of the cells. The majority of the fetal cells were weakly reactive; by 2 weeks of age maximal reactions were found in nearly all cells. In some sections, there was a suggestion of stronger reactions in large, corner cells, but this was not a consistent finding.

Although the intensity and/or frequency of staining increased with age in each histochemical reaction, no change in the pattern of localization was noted.

IV. DISCUSSION

Consistently weak or absent activity for these selected lysosomal enzymes was found in the fetal and newborn rat lung. In contrast, high levels of a variety of glycolytic and oxidative enzymes have been reported at these ages in the mouse.[11] The present results with cytochrome oxidase confirm this previous finding and suggest that the difference in species is not as important as the diversity of biochemical systems. The present results in adult animals also confirm the careful studies of Sorokin[12] and Goldfischer and co-workers[13] with respect to the localization of hydrolases in the type II cell (great alveolar cell, corner cell, granular pneumonocyte) and alveolar macrophage (phagocyte pneumonocyte). The paucity of macrophages in these sections is not surprising considering the absence of pathological lesions and the relatively small area sampled. There is no apparent explanation for the absence of reactivity in many type II epithelial cells.

The several morphologic cell types in pulmonary tissue are now well appreciated. Concurrently, there has been an increasing understanding of the lung as a metabolic organ with the capacity for synthesis and degradation of common as well as relatively specialized compounds.[14] Enzyme histochemistry is particularly useful in localizing metabolic pathways in the lung, since only the macrophage can be isolated for study with biochemical methods.

Is the finding of similar hydrolase activity in macrophages and type II cells representative of other basic similarities? While the question cannot be answered with complete confidence, available data emphasize distinct differences between them. Each has distinguishing ultrastructural characteristics; epithelial cells arise by local proliferation,[15] whereas the majority of pulmonary macrophages are of bone marrow origin.[16] There is considerable circumstantial evidence for the role of the type II cell in the production and secretion of lung surfactant, the surface tension - lowering phospholipid that prevents atelectasis in a normal lung,[17] but these cells lack the avid phagocytic activity of alveolar macrophages.*

* Esterly, J.R.; Faulkner, C.S. The granular pneumonocyte: Absence of phagocytic activity. Unpublished data.

It is tempting to speculate on the relationship between the minimal lysosomal enzyme activity in newborn animals and their susceptibility to infection because of the mechanism of these enzymes in host defense. Recent studies, however, suggest that deficient opsonins in neonatal serum may be a primary factor in susceptibility in this age group.[18]

Finally, it is of interest that no significant enzymatic activity was found in interstitial mononuclear cells in the alveolar septa. These cells are considered to be closely related to alveolar macrophages, and a varying proportion demonstrate phagocytosis. There may be several explanations, such as enzymatic activation by diapedesis into alveoli or an increase to stainable enzymatic concentrations only during contact with the airway. The most likely possibility, however, is the inability to identify definitely the majority of interstitial mononuclear cells as such in even the most satisfactory frozen sections.

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13. ABSTRACT

Histochemical methods were used to study the development of representative enzymes associated with lysosomal activity in sections of the lung from late fetal, neonatal, and adult rats. Staining for β-glucuronidase and acid phosphatase was present in the fetus and following birth, and the number and intensity of reactive cells increased to adult levels by 7 to 10 days of life. Reactions for β-galactosidase were not found in newborn animals, but moderate staining was seen by the 14th day. In contrast to these lysosomal enzymes, high levels of cytochrome oxidase staining were found in all specimens and no age-dependent changes were seen Acid phosphatase activity was present in nearly all cells in the adult lung, but the indolyl reactions for the glycosidases were localized to single cells. In spite of this resolution, it was not possible to be certain of the identity of many of the reactive cells. The majority appeared to be type II epithelial cells. Alveolar macrophages were usually reactive but relatively few such cells were found, especially in the neonatal specimens. The results of this and other studies indicate that these cells have many common metabolic pathways. Other data, however, suggest a dissimilar origin and function of type II cells and macrophages.

14. Key Words

*Enzymes	*Phagocytes	Rats	Infection
*Lung	Alveoli pulmonis	Hydrolases	

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